EXPERIMENTAL ATTEMPTS TO TRANSMIT INFECTIOUS MONONUCLEOSIS TO MAN*

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Knowledge concerning the causative agent of infectious mononucleosis has been sharply limited by the failure to reproduce all the features of the disease in the experimental animal. A comprehensive study evaluating the results of previous investigations of its causation and the attempts to transmit it to many laboratory animals, including the monkey, has been published by Wising[14] and will not be discussed here.

The purpose of this paper is to review briefly the experiments in which transmission of infectious mononucleosis to human volunteers has been attempted, and to present the results of similar investigations carried out in this laboratory.

Previous investigations

The results of previous efforts to reproduce infectious mononucleosis in human subjects are summarized in table 1. It will be observed that of 40 individual attempts utilizing a wide variety of inocula only two experiments were apparently successful: the first of these was reported in 1940 by Sohiér, Lépine, and Sautter,[10] who used whole blood obtained from a monkey inoculated 16 days before with similar material from a patient with infectious mononucleosis. The subject was injected intramuscularly with 6.0 cc. of this inoculum and subsequently developed suggestive hematological and serological characteristics of the disease even though he had few symptoms and no fever. The second successful attempt was published by Wising[13] in 1942. In this instance, 250 cc. of heparinized whole blood from a patient in the acute phase of the disease were injected intravenously into a human subject and resulted in an illness clinically, hematologically, and pathologically indistinguishable from infectious mononucleosis. The serum of this volunteer showed, however, only a very slight rise in the agglutination titer against sheep red blood cells.

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TABLE 1

SUMMARY OF PREVIOUS ATTEMPTS TO TRANSMIT INFECTIOUS MONONUCLEOSIS TO MAN

Authors	*Date*	*No. of Exper.*	*INOCULUM*				*Result*
			Type	*Treatment*	*Amount*	*Route*	
					c.c.		
Weipert and Woodruff[12]	1937	3	Nasal washings	Filtered	3.0	IN*	Negative
Sohiér, Lépine and Sautter[10]	1940	1	Whole monkey blood	None	6.0	IM	Positive
Wising[13]	1942	6	Lymph node suspension	Filtered and unfiltered	2-4	SC, IN, SM, IM, Topical	Negative
		2	Plasma	Filtered	100	IV	Negative
				Filtered	180	IV	Negative
		3	Whole blood	None	100	IV	Negative
				None	250	IV	Positive
				None	350	IV	Negative
Bang[2]	1943	3	Nose, sinus, tonsil washings	Filtered	2-4	Oral	Negative
		5	Lymph node suspension	Filtered	1-2	SC, Oral	Negative
		9	Whole blood	None	20-325	IM and/ or IV	Negative
Julianelle, Bierbaum, and Moore[7]	1944	2	Throat washings	Filtered	—	Oral	Negative
		2	Lymph node suspension	Filtered	—	IN, IM	Negative
Havens[6]	1945	2	Serum	Frozen	0.5	Oral, IN	1 Uncertain
		2	Serum	Frozen	0.5	SC, IC	1 Uncertain

* IN — Intranasal
IM — Intramuscular
IC — Intracutaneous
IV — Intravenous
SC — Subcutaneous
SM — Submucosal (Mouth)

It should be pointed out that only one of 3 subjects inoculated with whole blood by Wising[13] showed evidence of the disease and that Bang[2] working concurrently with similar material failed to achieve successful transmission in nine experiments carried out on human volunteers.

Experimental

During the past six or eight years there have been repeated attempts in this laboratory to transmit infectious mononucleosis to monkeys,[11] chimpanzees,[6] and to man.

In the present report mention is made of four unpublished attempts carried out by Havens[6] in this laboratory to transmit the disease to human volunteers, as well as subsequent experiments carried out by the author.

Donors: Data concerning these are shown in table 2. The donors with infectious mononucleosis represented patients in the New Haven

TABLE 2

DONORS USED FOR TRANSMISSION EXPERIMENTS

Donor	*Age*	*Day inoc. obtained*	*Donor's Signs of Inf. Mono.*				*Remarks*
			Day of Disease	*Per cent Lymphs*	*Sheep RBC titer*	*Fever*	
1.	5	6	4	43		+	Severe, GGE, sore throat,
			6		1:320	+	Enlarged liver and spleen
2.	23	3	3	24	Neg.	+	Severe, GGE, sore throat
			7	59		+	Exact sheep RBC titer not available.
			14	93	Pos.	0	
3.	22	16*	21*	60	Neg.	0	Volunteers previously given serum from donor 2 which resulted in sore throat, GGE
4.	22	16*	25*	64	Neg.	0	
5.	20	3	3	34	1:10	+	Suspect Inf. Mono. Later found
			10	42	1:10	0	to have only an U.R.I.
6.	20	4	4	67	1:640	+	Mild. Ambulatory. Slight fever.
			17	43	1:640	0	Sore throat. Moderate GGE.
			66		1:40	0	
7.	2	7	7	76	1:20	+	Severe. Rash, GGE, Pharyngitis,
			9	70	1:10	0	Splenomegaly.
			15		1:20	0	
8.	23	10	9	80	1:2560	+	Severe. Vincent's stomatitis,
			14	52	1:1280	0	GGE. High fever.
			30		1:320	0	
9.	20	2	2	53	1:40	+	Moderately severe. Sore throat
			11		1:2560	0	GGE. Splenomegaly.
10.	19	4	4	50	1:640	+	Mild. Sore throat. Little fever.
			8		1:160	0	
11.	23	8	9		1:1280	+	Severe. Peritonsillar abscess
			11	23	1:1280	+	GGE, High fever, Rash.
			15	68	1:2560	0	

* Day after inoculation.
GGE = Generalized Glandular Enlargement.

Hospital, from the Yale University Department of Health, and one "private" patient seen at home. Eleven donors were used. Donor 2 had been employed in earlier experiments in this laboratory[6] and his serum had resulted previously in suggestive results in 2 or 4 volunteers. Serum from these 2 subjects in whom questionable transmission had occurred was obtained on the 16th day after inoculation and utilized in the present experiments; these volunteers are designated as donors 3 and 4. Donor 5, as was later shown, had only an upper respiratory infection whose initial complaints suggested early infectious mononucleosis, and donor 7, though typical in all other respects, never developed a significant sheep red cell agglutination titer. All other donors had unquestionable evidence of infectious mononucleosis.

Subjects: Seventeen healthy male volunteers, aged from 19 to 25, were employed as the experimental subjects in an institution in which no known case of infectious mononucleosis had occurred for at least a year prior to the experiment and in which no naturally occurring case was observed during the period of experimentation. The subjects had no contact with the outside community but were not segregated from the others in the institution unless symptoms appeared, at which time they were isolated in the infirmary.

Examination of subjects: A careful history was taken on each volunteer prior to inoculation to exclude those with recent illnesses suggestive of a prior attack of infectious mononucleosis, and a complete physical examination was carried out with special attention to the status of the lymphatic system. Each volunteer was examined at least once a week during the month after inoculation, and any symptoms appearing for at least another month thereafter were promptly investigated.

Inocula: Unfiltered throat washings, serum, and citrated whole blood were employed in the transmission experiments and were obtained from the donors listed in table 2 and from one normal subject. Serum and throat washings were kept frozen with dry ice at -76° C. for periods ranging from 1 month to 2 years until just before use. Throat washings were shown to contain no hemolytic streptococci before they were utilized, and pulmonary tuberculosis was excluded in the donor by x-ray examination. Citrated whole blood was kept refrigerated until just before injection, a period never longer than 2.5 hours. No donor had a positive blood culture.

Laboratory work: Urinanalysis, complete blood counts, bromsulfalein dye excretion test, and a Mazzini test for syphilis were performed prior to inoculation. A total leukocyte count and a differential blood

count, using Wright's stain and enumerating 200 cells on a cover slip preparation, were made on each volunteer at approximately 3-day intervals or daily if symptoms appeared. The following tests were performed prior to inoculation and at weekly intervals thereafter: sheep RBC agglutination,[1] sedimentation rate (Cutler), urinary urobilinogen,[4] cephalin-cholesterol flocculation test,[5] and the thymol turbidity test.[8] In addition, alkaline serum phosphatase,[3] and total serum bilirubin including the one-minute determination[9] were ascertained weekly on the volunteers for the last 11 experiments.

Two volunteers were inoculated with normal human serum for control purposes and followed by the same laboratory tests as were the others.

Results

Twenty-one experiments were conducted on 17 volunteers and the results are shown in table 3. In no instance did any volunteer develop definite evidence of the experimental transmission of infectious mononucleosis. However, four subjects showed symptoms, signs, or changes in laboratory tests that were faintly suggestive and these can be summarized briefly as follows:

Volunteer 3, experiment 3, on the 18th day following oral and intranasal inoculation with serum developed a moderately severe sore throat and tenderness of the glands in the neck. Physical examination revealed only pharyngeal injection without exudate, and some enlargement of the posterior cervical and left axillary lymph nodes. These symptoms were of short duration and there was no fever. Laboratory work: there was no change in total leukocyte count but the lymphocytes rose to 46 per cent by the 27th day and there were 12 per cent monocytes many of which appeared atypical. There was no alteration in heterophile antibody titer, sedimentation rate, or liver function tests during this period.

Volunteer 6, experiment 6, on the 66th day following parenteral inoculation with serum developed a severe pharyngitis and a fever reaching 103° F., but showed no other clinical, hematological, or serological evidence of infectious mononucleosis.

Volunteer 11, experiment 11, 13 days after oral and intranasal inoculation with unfiltered throat washings complained of sore throat, chilliness, and frontal headache. Physical examination revealed an injected pharynx, but there was no adenopathy. Laboratory work: on the 17th day after inoculation there was a lymphocytosis of 64 per cent and many of these cells were atypical and resembled those seen

TABLE 3

RESULTS OF ATTEMPTS TO TRANSMIT INFECTIOUS MONONUCLEOSIS TO MAN

Exp. No.	Volun. No.	Donor No.	Inoculum			Findings in volunteers				Final Result
						Per cent Mononuclears*				
			Type	Amount In cc.	Route	Pre-IN	Post-IN	Day	Symptoms and signs	
1	1	1	Serum	1.5	SC, IC	35	52	14	None	Neg
2	2	1	Serum	1.5	SC, IC	39	57	17	None	Neg
3	3	1	Serum	0.5	ORAL, IN	38	58	28	Sore throat, Adenopathy	Neg
4	4	1	Serum	0.5	ORAL, IN	34	48	18	None	Neg
5	5	2	Serum	1.0	SC, IC	41	62	27	None	Neg
6	6	2	Serum	1.0	SC, IC	42	52	27	Pharyngitis 66th day	Neg
7	7	3 & 4	Serum	1.0	SC, IC	41	49	16	None	Neg
8	8	3 & 4	Serum	1.0	SC, IC	39	47	16	Sore throat 23rd day	Neg
9	9	5	Whole Blood	5.0	IM	34	53	27	URI 2nd day	Neg
10	10	5	Whole Blood	5.0	IM	27	33	17	URI 2nd day	Neg
11	11	6	Unf. Th. Wa.	10.0	ORAL, IN	40	65	17	Pharyngitis 18th day	Neg
12	12	6	Unf. Th. Wa.	10.0	ORAL, IN	28	48	18	Pharyngitis 2nd day	Neg
13	13	7	Whole blood	5.0	IM	41	50	10	None	Neg
14	14	7	Whole blood	5.0	IM	32	46	10	None	Neg
15	15	8	Whole blood	5.0	IM	43	50	4	None	Neg
16	16	8	Whole blood	5.0	IM	35	35	31	None	Neg
17	17	9	Unf. Th. Wa.	10.0	ORAL, IN	46	59	32	None	Neg
18	13	1	Unf. Th. Wa.	10.0	ORAL, IN	38	48	36	None	Neg
19	14	8	Unf. Th. Wa.	10.0	ORAL, IN	38	39	23	None	Neg
20	1	10	Unf. Th. Wa.	10.0	ORAL, IN	29	?		None	Neg
21	7	11	Unf. Th. Wa.	10.0	ORAL, IN	39	58	6	None	Neg

* Mononuclears = Lymphocytes + Monocytes.
Unf. Th. Wa. = Unfiltered Throat Washings.
Pre-IN and Post-IN refer to the highest counts observed before and after inoculation.
DAY refers to the day after inoculation on which highest count occurred.
SC = Subcutaneous; IC = Intracutaneous; IN = Intranasal; IM = Intramuscular.
Circumstances prevented laboratory work in Exp. 20.

in infectious mononucleosis. This blood picture persisted approximately 4 days and there was no other significant change in laboratory tests.

Volunteer 13, experiment 18, remained asymptomatic following oral and intranasal inoculation with unfiltered throat washings, but on the 14th day the serum from this subject showed a 3-plus cephalin-cholesterol flocculation and a slight increase in the value for alkaline serum phosphatase above the normal limit. In addition, on the 17th day his blood showed a 40 per cent lymphocyte count with 5 per cent atypical forms. There was no change in total leukocyte count or in other laboratory tests, and the abnormalities in liver function tests and hematological picture returned to normal levels within a week.

Discussion

It has been reported in this paper that 21 experiments attempting the transmission of infectious mononucleosis to human volunteers have been carried out in this laboratory using serum, whole blood, and unfiltered throat washings without successful transmission of the disease in a single instance. Suggestive results as indicated by pharyngitis, lymphadenopathy, lymphocytosis, and the appearance of atypical lymphocytes occurred in a few inoculated subjects but the transient nature of these signs and symptoms and the absence of other supporting evidence make their occurrence only remotely suggestive of the transmission of infectious mononucleosis. These findings are in keeping for the most part with the previous experience of others and with that of Havens[6] in this laboratory.

Assuming that a causative agent is present in the blood or throat of patients during the acute phase of infectious mononucleosis, our failure, as well as that of other investigators, to transmit the disease to human volunteers might be due to 2 factors: 1) the causative agent may be extremely labile and easily destroyed by freezing or other manipulation, 2) individual susceptibility may be low in the volunteers employed. Since apparently successful transmission has resulted from the immediate injection of whole blood as the inoculum in two instances, yet failed in many others, either or both of these factors might be operative.

Summary

Twenty-one experiments attempting the experimental transmission of infectious mononucleosis to 17 human volunteers have been carried out using serum, whole blood, and unfiltered throat washings.

In no instance was definite evidence of successful transmission obtained, but a few subjects showed suggestive signs or symptoms as indicated by sore throat, lymphadenopathy, or a hematological picture similar to that seen in infectious mononucleosis.

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